IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Richard BÉLIVEAU et al.
Serial number:	10/556,145
Filing date:	August 21, 2006
For:	COMPOUND AND METHOD FOR REGULATING PLASMINOGEN ACTIVATION AND CELL MIGRATION
Art Unit:	1643
Examiner:	GUSSOW, Anne
Agent:	Mark J. Nuell

DECLARATION UNDER 37 C.F.R. SEC. 1.132

I, Michel Demeule, do hereby declare and state as follows:

1. I received the degrees of DEC in Sciences from College Edouard Montpetit in 1982; Bachelor of Biochemistry from UQAM in 1986; Master of Chemistry from UQAM in 1988; and Doctor of Philosophy (Science) from Université de Montréal in 1992.

2. My academic background and experiences in the field of the present invention are listed on the enclosed *curriculum vitae*.

3. I am a Scientist at Angiochem Inc since June 2006.

4. I am an author of several scholarly publications as listed in my enclosed *curriculum vitae*.

5. I am an inventor in the present application; I have read and am thoroughly familiar with the contents of U.S. Patent Application Serial No. 10/556,145,

entitled "COMPOUND AND METHOD FOR REGULATING PLASMINOGEN ACTIVATION AND CELL MIGRATION", including the claims.

6. I have also read and understood the latest Official Action from the PTO dated October 17, 2007. In this Office Action, certain claims (36-38) were rejected for lack of enablement under 35 U.S.C. §112, first paragraph.

7. The following experiments had been performed from August 2005 to March 2007, under my supervision, to confirm efficacy of the method on cells not expressing p97 on their surface.

   The following experiment was conducted to evaluate the inhibition of tumor growth by a soluble form of melanotransferrin (sMTf).

   Background of the model: Our previous results demonstrated that a truncated and solubleform of melanotransferrin (sMTf) reduces the migration of cancer cells and angiogenesis (1-2). We are now presenting new experiments showing the efficacy of sMTf on the growth of subcutaneous tumors derived from human glioblastoma (U87 MG cells) and lung carcinoma (NCI H460 cells).

   Glioblastomas are characterized by a high proliferation rate, extensive angiogenesis, and marked local invasion that makes these tumors resistant to the conventional treatment modalities of surgery, chemotherapy and radiotherapy (3). Therefore, several attempts have been initiated to inhibit vascularization of experimental gliomas in order to establish novel therapeutical approaches. In terms of angiogenesis research, U87 glioma cell line has been utilized for various objectives (4-8). On the other hand, lung cancer is responsible for approximately one-third of all cancer-related deaths in the United States each year, killing more Americans than breast cancer, colon cancer, and prostate cancer combined (9). Non-small cell lung carcinoma is the most common histological cell type and often presents in an advanced stage. Despite several advances, chemotherapy has been largely ineffective and over 85% of patients with lung cancer eventually

succumb to the disease (10). NCI H460 cell line is widely used to measure the efficacy of anticancer agents on non-small cell lung cancer (10-13).

On the basis of our previous observations, we hypothesized that sMTf would affect the motility of mMTf expressing cells whether their origins are endothelial or tumoral. We are clearly showing that sMTf inhibits angiogenesis in U87 MG-derived tumors, thus leading to the inhibition of tumor growth. Indeed, results shown here demonstrate that sMTf treatment interferes with the growth of subcutaneous tumors derived from U87 MG and NCI H460 cells. Furthermore, that sMTf cooperates or interacts with LRP in order to regulate plasmin generation at the cell surface and meddle in cancer cell growth (14).

References:

(1) Demeule M, Bertrand Y, Michaud-Levesque J, Jodoin J, Rolland Y, Gabathuler R, Béliveau R. 2003. Regulation of plasminogen activation: a role for melanotransferrin (p97) in cell migration. Blood 102(5): 1723.

(2) Michaud-Levesque J, Demeule M, Béliveau R. 2007. In vivo inhibition of angiogenesis by a soluble form of melanotransferrin. Carcinogenesis 28(2): 280.

(3) Giese, A., and Westphal, M. Glioma invasion in the central nervous system. eurosurgery, *39:* 235–250, 1996.

(4) Stan AC, Nemati MN, Pietsch T, Walter GF, Dietz H. 1995. In vivo inhibition of angiogenesis and growth of the human U-87 malignant glial tumor by treatment with an antibody against basic fibroblast growth factor. J. Neurosurg 82 : 1044.

(5) Cheng SY, Huang HJ, Nagane M, Ji XD, Wang D, Shih CC, Arap W, Huang CM, Cavenee WK. 1996. Suppression of glioblastoma angiogenicity and tumorigenicity by inhibition of endogenous expression of vascular endothelial growth factor. Proc Natl Acad Sci USA 93 : 8502.

(6) Bernsen HJ, Rijken PF, Peters JP, Bakker H, van der Kogel AJ. 1998. Delayed vascular changes after antiangiogenis therapy with antivascular endothelial growth factor antibodies in human glioma xenografts in nude mice. Neurosurgery 43 :570.

(7) Im SA, Gomez-Manzano C, Fueyo J, Liu TJ, Ke LD, Kim JS, Lee HY, Steck PA, Kyritsis AP, Yung WK. 1999. Antiangiogenesis treatment for gliomas : transfer of antisense-vascular endothelial growth factor inhibits tumor growth in vivo. Cancer Res 59 :895.

(8) Plate, K. H., and Risau, W. Angiogenesis in malignant gliomas. Glia, 15: 339-347, 1995.

(9) American Cancer Society. Key Statistics about lung cancer, 2007.

(10) Bunn P. A., Kelly K. New chemotherapeutic agents prolong survival and improve quality of life in non-small cell lung cancer: a review of the literature and future directions. Clin. Cancer Res., *5:* 1087-1100, 1998.

(11) Kraus-Berhier L, Jan M, Guilbaud N, Naze M, Pierré A, Atassi G. 2000. Histology and sensitivity to anticancer drugs of two human non-small cell lung carcinomas implanted in the pleural cavity of nude mice. Clin Cancer Res 6(1) : 297.

(12) Yang L, Mashima T, Sato S, Mochizuki M, Sakamoto H, Yamori T., Oh-Hara T, Tsuruo T. 2003. Predominant supression of apoptosome by inhibitor of apoptosis protein in non small cell lung cancer H460 cells : therapeutic effect of a novel polyarginine-conjugated Smac peptide. Cancer Res 63(19) : 6566.

(13) Chien W, Yin D, Gui D, Mori A, Frank JM, Said J, Kusuanco D, Marchevsky A, McKenna R, Koeffler HP. 2006. Suppression of cell proliferation and signaling transduction by connective tissue growth factor in non small cell lung cancer cells. Mol Cancer Res 4(8): 591.

(14) Michaud-Levesque J, Demeule M, Béliveau R. 2007. Plasminogen-dependant internalization of soluble melanotransferrin involves the low-density lipoprotein receptor-related protein and annexin II. Biol Chem 388(7): 747.

sMTf reduces the growth of glioblastoma and lung carcinoma subcutaneous tumors

The impact of sMTf administration on xenografts tumor growth was investigated. U-87 MG and NCI-H460 cells were inoculated into the right flank of nude mice whereas Alzet osmotic micro-pumps containing sMTf or Ringer/HEPES control solution were implanted in their left flank. In the glioblastoma model, control mice showed a tumor growth upon the fifth day of treatment, while mice treated with sMTf (2.5 mg/kg/day) display a tumor growth at 18 days of treatment. Interestingly, tumors from mice treated with sMTf 10 mg/kg/day do not present significant growth toward the study (Fig. 1). We observed a 41.3% regression in tumor volume for 3 out of 8 mice treated with sMTf 10 mg/kg/day. After 18 days of treatment, i.e. when the first tumor of control mice reached 1000 mm^3, the difference in tumor size between control and sMTf-treated mice was significant. We were able to determine a tumor growth inhibition of 73.8% and 91.8% in

sMTf-treated mice at 2.5 and 10 mg/kg/day, respectively (Table 1). In the lung carcinoma model, sMTf treatment (2.5 mg/kg/day) inhibits tumor growth for a 15-day period. After 18 days of treatment, mice treated with sMTf at 2.5 and 10 mg/kg/day have tumors with average volumes reaching 140 and 65 mm^3 respectively, compared to 642 mm^3 for tumors in the control group (Table 1). Furthermore, tumor growth inhibition was sustained for 18 days in mice treated with 10 mg/kg/day of sMTf (Fig. 1) and complete remission was observed in one case. Seven out of 9 mice treated with sMTf 10 mg/kg/day showed a regression in tumor size of about 50% when compared to the initial tumor volume. The corresponding tumor growth inhibition values reached 87.6% and 97.2% in mice treated with sMTf at 2.5 and 10 mg/kg/ day, respectively (Table 1).

Additionally, tumor growth inhibition by sMTf at 10 mg/kg/day is significantly different from that obtained at 2.5 mg/kg/day in both subcutaneous xenograft models. sMTf can thus be considered as an active antitumor protein. In both cases, sMTf treatment had no significant effect on mouse body weight and a gross necropsy revealed no physiological modifications.

Table 1 – Inhibition of tumor growth by sMTf

Tumor origin	sMTf dosage (mg/kg/day)	Volumes (mm^3)		Tumor growth inhibition (%)	Partial tumor regression (9–11 days)
		Initial	Final		
Glioblastoma	0	88 ± 32	591 ± 229	0	0/8
	2.5	92 ± 33	229 ± 120	73.8	1/8
	10	63 ± 7	104 ± 25	91.8[a]	3/8
Lung carcinoma	0	71 ± 29	642 ± 240	0	0/9
	2.5	69 ± 26	140 ± 69	87.6	5/9
	10	49 ± 15	65 ± 32	97.2[a]	7/9

Tumor cells from human glioblastoma (U-87 MG) and lung carcinoma (NCI-H460) were inoculated subcutaneously in Crl:CD-1®-nuBR nude mice. After 3 days, micro-osmotic pumps containing Ringer/HEPES control solution or sMTf were implanted as described in Materials and methods. The activity end points used to determine tumor inhibition is the median tumor volumes in mm^3 of the treated control groups. Median tumor volumes were determined on the first day treatment and at the end of the study – i.e. when the first mouse of the control group held a tumor of 1000 mm^3. Tumor growth inhibition by sMTf is expressed as percent of control. Animals bearing tumor with a final volume below its initial one are defined here as partial tumor regression.

[a] Results are significantly different when compared to sMTf 2.5 mg/kg dose, $p < 0.01$.

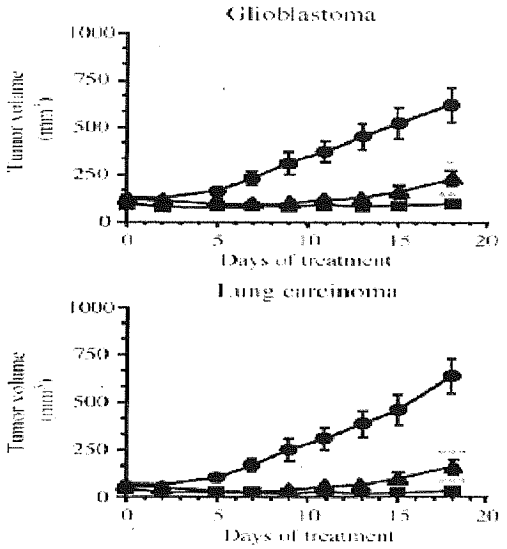

Fig. 1 – Inhibition of tumor growth by sMTf. Nude mice were subcutaneously inoculated with U-87 MG glioblastoma cells and NCI-H460 large lung carcinoma cells. At 3 days after cell inoculation, animals were randomly separated in three groups and Alzet micro-osmotic pumps filled with Ringer/ HEPES control solution (●) or sMTf at 2.5 (▲) and 10 mg/kg/ day (■) were implanted as described in Materials and methods. Experiments were performed on eight different animals for all conditions and the means±S.E. are shown. Statistically significant differences in tumor volumes are indicated by ** for $p < 0.005$ and * for $p < 0.001$ (Student's *t*-test) when compared to tumors from the control group.**

sMTf inhibits angiogenesis in subcutaneous glioblastoma development

In order to demonstrate that sMTf induced a reduction in tumor growth by interfering throughout the angiogenic process, heamoglobin (Hb) content was quantified in subcutaneous tumors derived from U-87 MG and NCI-H460 cells. The results demonstrated that Hb content was 2-fold higher in U-87 MG than in NCI-H460 tumors (Fig. 2A), indicating that angiogenesis in subcutaneous NCI-H460-derived tumor is much lower than in U-87 MG-derived ones. This led us to evaluate angiogenic development in U-87 MG-derived tumors, rather than in the lung carcinoma model. As soon as the first tumor from the control group reached 1000 mm^3, tumors were excised and the Hb content was measured. Fig. 2B demonstrates that sMTf treatment resulted in a reduction of Hb levels at both doses by approximately 50%. Additionally, mRNA expression of CD105, an endothelial cell marker from newly formed vessels, was greatly down regulated in sMTf-treated U-87 MG-derived tumors (Fig. 2C). However, the mRNA

expression of another endothelial cell marker, CD31 or PECAM, was unaffected by sMTf treatments when compared to the loading control β-tubulin (Fig. 2C). Western blot analysis also revealed that CD31 protein expression in U-87 MG-derived tumors was unchanged after sMTf treatments. The haemoglobin content and CD105 mRNA levels in U-87 MG-derived tumors show that sMTf exerts an anti-angiogenic activity during the development of subcutaneous glioblastoma.

Since angiogenesis is strongly induced by cytokines from tumor cells, levels of bFGF and VEGF mRNA were studied in tumor tissue derived from U-87 MG cells. The mRNA expression of these growth factors was unchanged in subcutaneous glioblastoma tumors treated with sMTf when compared to tumor tissue from the control group. These results indicate that although sMTf inhibits bFGF- and VEGF induced angiogenesis, the inhibition of angiogenesis by sMTf is not the result of a negative regulation in the expression of these growth factors in U-87 MG-derived tumors.

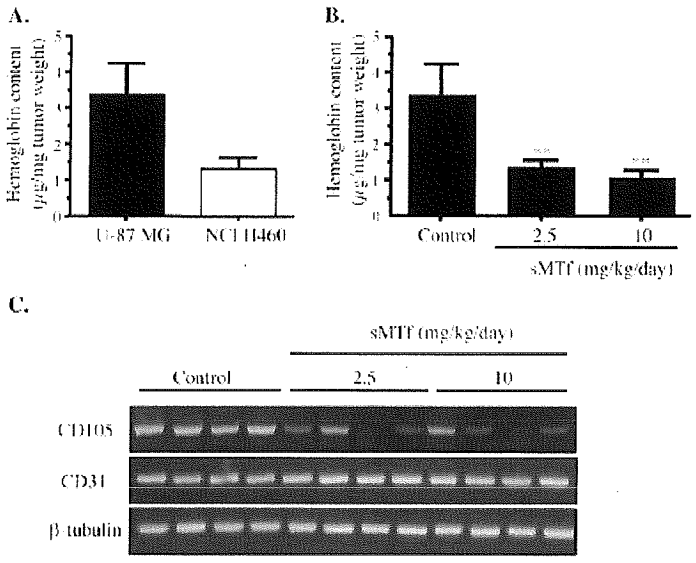

Fig. 2 – sMTf treatment reduces angiogenesis in vivo. Nude mice bearing tumors derived from U-87 MG and NCI-H460 cells were treated using Alzet micro-osmotic pumps containing with Ringer/HEPES control solution or sMTf (2.5 and 10 mg/kg). (A) Control tumors derived from U-87 MG and NCI-H460 cells with a volume of around 1000 mm^3 were excised and the hemoglobin contents were determined as described in Materials and methods. (B) When the first U-87 derived tumor from the control group reached 1000 mm^3, control and sMTf-treated tumors were excised and lyophilized to evaluate their hemoglobin content. (C) mRNA expression of CD105 (endoglin) and CD31 (PECAM) were also assessed on U-87 MG-derived tumors treated or not with sMTf (2.5 and 10 mg/kg). RT–PCR analysis of β-tubulin was used as a loading control. Experiments were performed on eight different animals for all conditions and the means ± S.E. are shown. Statistically significant differences between hemoglobin contents are indicated by ** for $p < 0.005$ (Student's *t*-test) when compared to the hemoglobin content in tumors from the control group.

Effects of sMTf on tumor cell-invasive capabilities

We decided to extend this analysis to U-87 MG and NCI-H460 cells. While LRP and u-PAR protein expression were unchanged in sMTf-treated U-87 MG glioma cells, the same treatment induced a significant reduction in the expression of both receptors in NCI-H460 large lung carcinoma cells. In fact, LRP and u-PAR could not be detected in these cells after sMTf treatment, as shown in Fig. 3A. In agreement with prior results, the treatment of HMEC-1 endothelial cells with sMTf also resulted in a great reduction of both LRP and u-PAR protein expression when compared to the loading control GAPDH (Fig. 3A). It is to noted that mMTf expression was unaffected by sMTf in NCI-H460 and HMEC-1 cells (Fig. 3A–B). In addition, endogenous mMTf levels were under the detectable limit in U-87 MG cells.

Several members of the LDLR family, including LRP, are involved in the regulation of u-PA/u-PAR activity. Even though previous studies report modulation of these receptors during the regulation of the u-PA/u-PAR system, LRP mRNA levels were unaffected in NCI-H460 and HMEC-1 cells treated with sMTf under our experimental conditions (Fig. 3B).

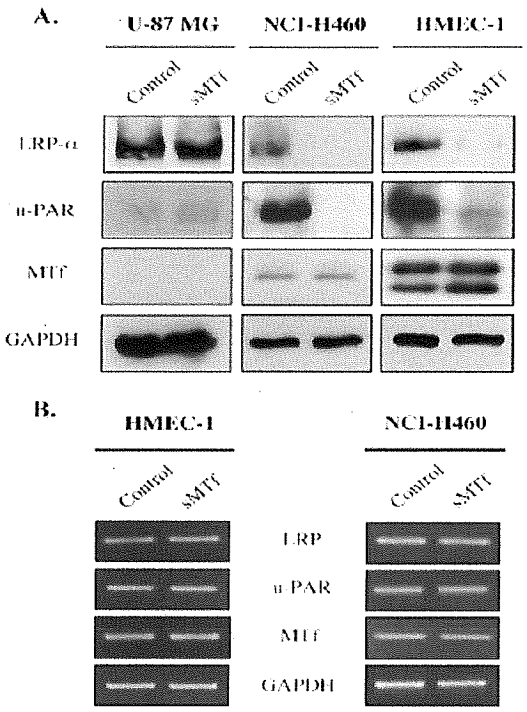

Fig. 3 – sMTf treatment affects the u-PAR/LRP system in endothelial and large lung carcinoma cells. (A) Protein expression of u-PAR and LRP were studied by Western blot in cells from human glioblastoma (U-87 MG), large lung carcinoma (NCI-H460) and in microvascular endothelial cells (HMEC-1) treated or not with 100 nM sMTf for 18 h. (B) mRNA expression of u-PAR and LRP was also studied by semi-quantitative PCR in U-87 MG, NCI-H460 and HMEC-1 cells treated or not with sMTf. GAPDH expression was used as a loading control. Results are representative of three independent experiments.

siRNA-mediated MTf knockdown reduces TF-induced lung metatstases in nude mice

The Plg system is considered as the primary effector of cell invasion. During cell invasion, this system mediates destruction of the extracellular matrix by fibrinolysis. In addition, tissue factor (TF), an initiator of the extrinsic coagulation cascade, is also expressed in a wide range of cancer cells and plays an important role in invasion and cancer progression. For example, by promoting fibrin deposition, TF plays a key role in the metastatic process and angiogenesis such of lung cancers.

To determine whether MTf is involved during *in vivo* TF-induced SK-Mel-28 melanoma invasion into lung, metabolically radiolabeled SK-Mel-28 cells were injected intravenously into mice that were preteated (or not) with TF. The melanoma cell invasion into the organs was assessed by measuring the infiltration of SK-Mel-28 cells into these organs in untreated and TF-treated mice. [^{3}H]-thymidine-labeled SK-Mel-28 cell accumulation was observed to increase in lung by threefold whereas the kidney, heart, and brain accumulation was similar to that in the control animals. Altogether, these results demonstrated a correlation between the levels of [^{125}I]-fibrin deposition and of SK-Mel-28 cell accumulation in the lung (Fig. 4B). However, siRNA-mediated MTf knockdown reduces by about 80% the lung invasion by TF-induced SK-Mel-28 cells in nude mice (Fig. 4C). This reduction of SK-Mel-28 cell metastasis in the lung observed with MTf-silenced cells indicates that MTf is involved during melanoma cell lung invasion.

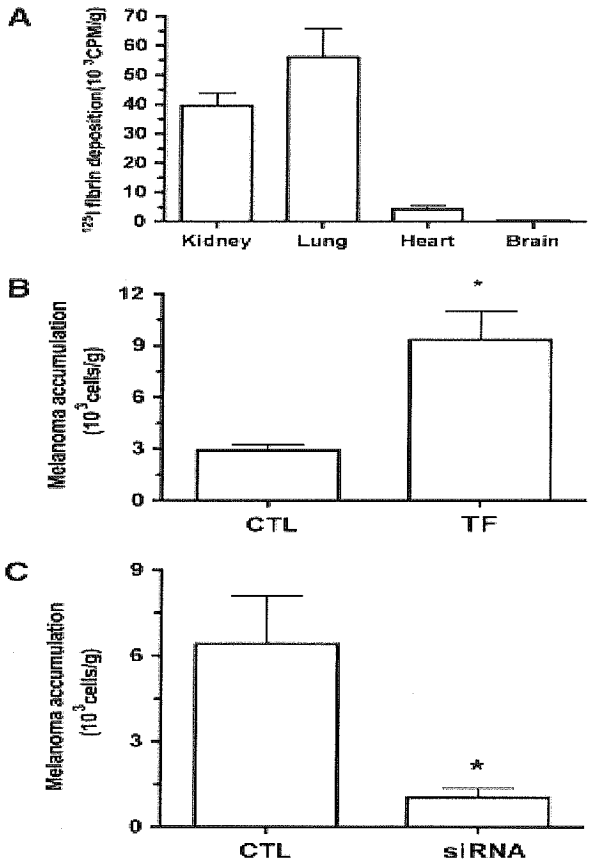

Fig. 4. siRNA-mediated MTf knockdown reduces TF-induced lung metastases in nude mice. (A) Fibrin clot deposition was performed in mice. Mice were treated with vehicle or with TF by i.v. injection. After 5 min [^{125}I]-fibrinogen was injected and [^{125}I]-fibrin deposition was detected at 20 min using a gamma counter. Several organs (kidney, lung, heart, and liver) were excised to monitor fibrin deposition. Data represent means ± SEM of five different experiments. (B) Cultured human melanoma SK-MEL-28 cells were radiolabeled with [H^3]-thymidine for 72 h. TF and cells were injected into nude mice via the tail vein. Radiolabeled SK-Mel-28 cells were measured in the lung with or without TF injection. Lung-associated radioactivity was determined for controls and for TF-induced SK-Mel-28 metastasis. Data represent means ± SEM of five different experiments. (C) Effect of siRNA-mediated MTf knockdown on SK-Mel-28 cells lung metastasis. Lung-associated radioactivity was determined in control and MTf-silenced SK-Mel-28 metastasis. Data represent means ± SEM of five different experiments. Statistically significant differences from control values are indicated by $^*P < 0.05$ (Student's t test).

bFGF-induced neovascularization involves the PA/plasmin system

We used the Matrigel™ plug neovascularization assay to study the effect of MTf on the modulation of *in vivo* angiogenesis. This assay permits the determination of the extent of angiogenesis into the Matrigel™ implant by direct measurement of the amount of Hb present in the implant. Analysis of the Matrigel™ implants that

did not contain growth factor showed very low pink hue coloration (Hb content), indicating that Matrigel™ itself was not angiogenic (Figure 5; upper panel). In contrast to Matrigel™ control condition, Matrigel™ implants containing bFGF showed strong neovascularization (Figure 5; upper panel). In the presence of bFGF alone, the amount of Hb was increased by 3-fold over the level found in control plugs containing no added growth factor (Figure 5; lower panel). The implication of the PA/plasmin system during the *in vivo* Matrigel™ plug neovascularization induced by bFGF was determined by adding into the implant the plasmin(ogen) inhibitor εACA during the assay. Lysine derivatives, such as εACA, are effective inhibitors of the PA/plasmin system. The addition of εACA (35 mg/ml) into the plug strongly inhibited by ~80% the angiogenic response elicited by bFGF, as indicated by the lower implant vascularization (Figure 5; upper panel) and lower Hb content (Figure 5; lower panel). These results show that plasmin generation from plasminogen is involved during bFGF-induced *in vivo* angiogenesis, indicating that this model is suitable for studying the impact of MTf on *in vivo* angiogenesis since MTf modulates plasmin generation by PA from plasminogen.

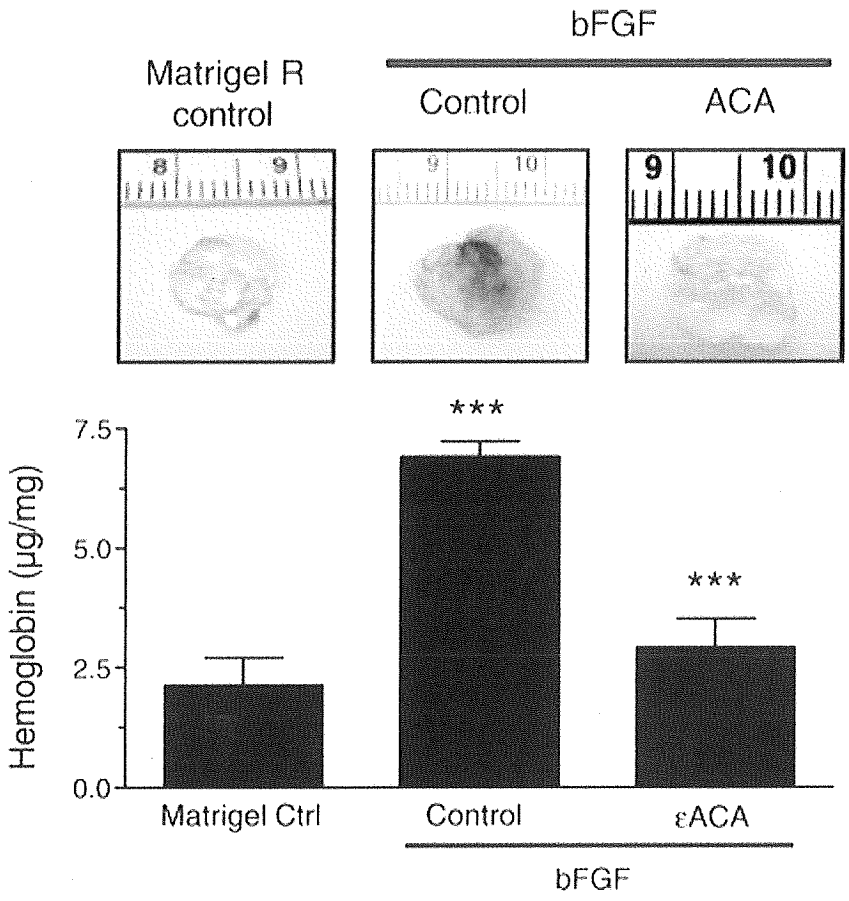

Fig. 5 bFGF-induced neovascularization involves the PA/plasmin system. bFGF-induced Matrigel™ plug *in vivo* angiogenesis assay containing, or lacking, εACA in nude mice, as described in the Materials and methods section. Photos obtained from representative samples are shown in the upper panel. Hb content from Matrigel™ implants are shown in the lower panel. Results in the lower panel are expressed as Hb content in dried Matrigel™ implant (µg Hb/mg dried implant). $n = 12$ for both control conditions, $n = 5$ for εACA condition and the means ± SE are shown. Statistically significant differences compared with respective control conditions are indicated as follows: $***P < 0.001$ (Student's *t*-test).

Subcutaneous, systemic truncated sMTf treatments inhibit bFGF- and VEGF-induced neovascularization

Given the important role of plasmin, a protein like MTf that targets the formation of plasmin and acts on the invasiveness capacity of EC and SK-Mel-28 cells as well as on EC tubulogenesis might be expected to affect *in vivo* angiogenesis. To test this hypothesis, Matrigel™ implant neovascularization was stimulated by bFGF and nude mice were treated systemically four times (Days 0, 2, 4 and 6 post-implantation; s.c. injection) with either truncated sMTf (10, 20 and 40 mg/kg; total treatment) or control R/H (Figure 6A and B). In the presence of

bFGF alone, there was a robust angiogenic response as indicated by the strong pink hue distributed throughout the plug (Figure 6A). To obtain a more quantitative analysis, the Hb content within each plug was measured to assess the angiogenic index (Figure 6B). When mice were treated with 10 mg/kg truncated sMTf, the amount of vascular development was reduced, whereas at 40 mg/kg the inhibition of vascularization was maximal, with a median effective dose (ED_{50}) at 14 mg/kg (Figure 6A and B). Furthermore, to show the generality of the truncated sMTf-mediated angiogenesis inhibition, we characterized the effect of truncated sMTf s.c treatment on *in vivo* Matrigel™ plug assay in the presence of another standard proangiogenic cytokine, for example, VEGF (Figure 6C). In contrast to Matrigel™ control condition, Matrigel™ implants containing VEGF showed strong neovascularization (Figure 6C; upper panel). In fact, the amount of Hb was increased by 3.5-fold in the presence of VEGF alone compared with control implants containing no added growth factor (Figure 6C; lower panel). When mice were treated systemically four times (Days 0, 2, 4 and 6 post-implantation; s.c. injection) with truncated sMTf (20 mg/kg; total treatment), the amount of vascular development within the Matrigel™ implant was significantly reduced by 68% (Figure 6C; lower panel). These results indicate that truncated sMTf inhibits both bFGF- and VEGF-induced angiogenesis, suggesting that s.c. administration of truncated sMTf provides a novel therapeutic strategy for the treatment of angiogenesis-related disorders.

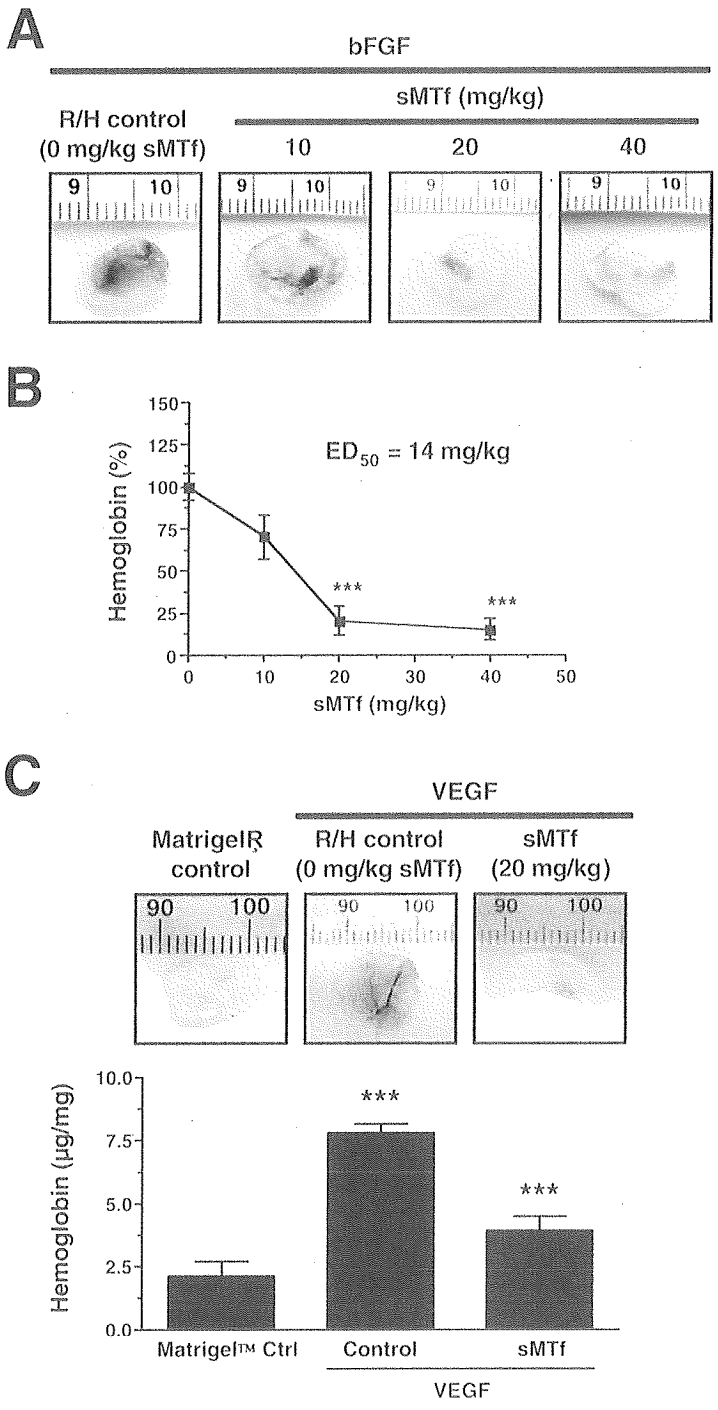

Fig. 6 Subcutaneous, systemic truncated sMTf treatment inhibits bFGF- and VEGF-induced neovascularization. bFGF- and VEGF-induced Matrigel™ plug *in vivo* angiogenesis assay in nude mice, as described in the Materials and methods section. (**A**) bFGF-induced Matrigel™ plug *in vivo* angiogenesis assay in nude mice treated systemically for 7 days with repeated s.c. injections of either R/H control solution or truncated sMTf (10, 20 and 40 mg/kg/week), as described in the Materials and methods section. Photos obtained from representative samples are shown. (**B**) Hb content from Matrigel™ implants containing bFGF in nude mice treated systemically for 7 days with repeated s.c. injections of truncated sMTf. Results were corrected for background Hb content measured under the Matrigel™ control condition and expressed as percentage inhibition compared

with R/H control condition. $n = 10$ for R/H control condition; $n = 4$ for each other conditions and the means ± SE are shown. (**C**) VEGF-induced Matrigel™ plug *in vivo* angiogenesis assay in nude mice treated systemically for 7 days with repeated s.c. injections of either R/H control solution or truncated sMTf (20 mg/kg/week), as described in the Materials and methods section. Photos obtained from representative samples are shown in the upper panel. Hb content from Matrigel™ implants are shown in the lower panel. Results in the lower panel are expressed as Hb content in dried Matrigel™ implant (μg Hb/mg dried implant). $n = 12$ for Matrigel™ control condition, $n = 5$ for each other conditions and the means ± SE are shown. Statistically significant differences compared with respective control conditions are indicated as follows: $***P < 0.001$ (Student's *t*-test).

MTf-expressing cells stimulate neovascularization, involvement of the PA/plasmin system

Since MTf overexpression stimulates cell motility, migration and invasion and MTf-expressing cell conditioned media stimulate *in vitro* HUVEC tubulogenesis, we determine the effects of endogenous MTf protein expression on *in vivo* angiogenesis. Thus, we examined the impact of CHO (control and MTf-transfected) and SK-Mel-28 melanoma cells within the implant on *in vivo* angiogenesis using the Matrigel™ plug neovascularization assay (Figure 7A). Quantitative analysis of angiogenesis indicated that MTf-expressing cells (MTf-transfected CHO and SK-Mel-28) induced by ~6-fold the neovascularization of the Matrigel™ implants, as demonstrated by the higher Hb content, whereas CHO control cell had no significant impact on Matrigel™ Hb content (Figure 7A; lower panel). In addition, MTf expression in CHO and SK-Mel-28 cells was unchanged following bFGF treatment. Also, bFGF secretion in CHO cells was unaffected by MTf transfection. These results show that MTf expression in CHO and SK-Mel-28 cells contribute to the increased angiogenic response in the *in vivo* Matrigel™ plug neovascularization assay. Moreover, we also determined the effect of εACA into the implant on the neovascularization induced by SK-Mel-28 cells (Figure 7B). The presence of εACA (35 mg/ml) into the plug strongly inhibited by ~75% the angiogenic response elicited by SK-Mel-28 cells, as indicated by the lower implant vascularization (Figure 7B; upper panel) and lower Hb content (Figure 7B; lower panel). These results indicate that the PA/plasmin system is involved during SK-Mel-28-induced *in vivo* angiogenesis.

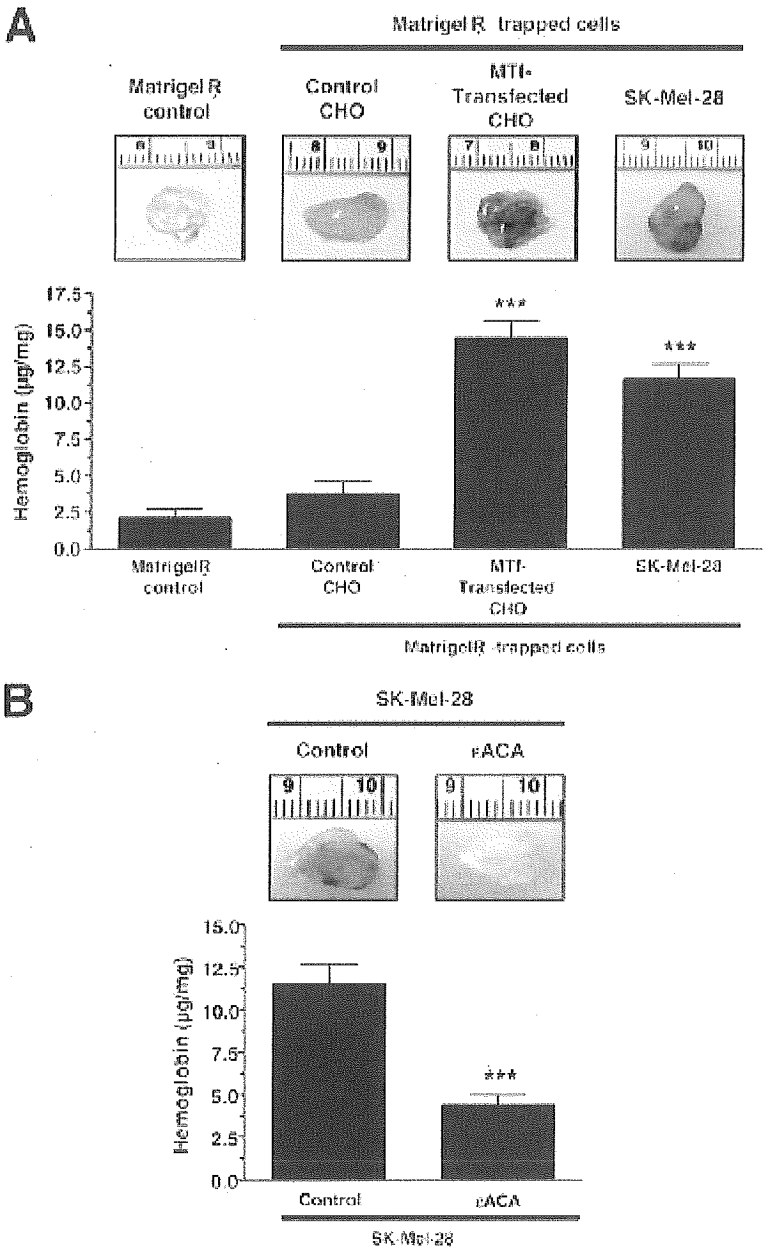

Fig. 7 MTf-expressing cells stimulate neovascularization, involvement of the PA/plasmin system. (**A**) Cell-induced Matrigel™ plug *in vivo* angiogenesis in nude mice, as described in the Materials and methods section. Photos obtained from representative samples are shown in the upper panel. Hb content from Matrigel™ implants containing, or lacking, CHO (control and MTf-transfected) and SK-Mel-28 cells are shown in the lower panel. $n = 12$ for Matrigel™ control condition, $n = 5$ for CHO cells conditions, $n = 7$ for SK-Mel-28 condition, (**B**) SK-Mel-28-induced Matrigel™ plug *in vivo* angiogenesis assay containing, or lacking, εACA in nude mice, as described in the Materials and methods section. Photos obtained from representative samples are shown in the upper panel. Hb content from Matrigel™ implants are shown in the lower panel. Results in the lower panel are expressed as Hb content in dried Matrigel™ implant (µg Hb/mg dried implant). $n = 7$ for SK-Mel-28 control condition, $n = 5$ for SK-Mel-28 + εACA condition and the means ± SE are shown. Statistically significant differences compared with respective control conditions are indicated as follows: $***P < 0.001$ (Student's *t*-test).

L235 inhibits SK-Mel-28-induced neovascularization

The mAb L235 was used to determine whether cell-induced Matrigel™ implant *in vivo* neovascularization was directly dependent on plasminogen activation induced by the MTf expression in SK-Mel-28 tumor cell. Thus, we added various concentrations of mAb L235 into the Matrigel™ implants containing SK-Mel-28 cells (Figure 8A and B). The addition of L235 mAb into the Matrigel™ inhibited, in a concentration-dependent manner, the SK-Mel-28-induced neovascularization and reached a maximal angiogenesis inhibition of 70% at 100 nM. The ED_{50} calculated for the inhibition of angiogenesis by L235 is ~46 nM (Figure 8B). Because the mAb L235 does not cross-react with other species than human, this mAb only binds to MTf expressed by the SK-Mel-28 melanoma cells. Since L235 reduces MTf-induced plasminogen activation at the cell surface and specifically inhibits SK-Mel-28-induced *in vivo* neovascularization of the Matrigel™ implants, these results confirmed that MTf overexpression in these melanoma cells leads to an increased angiogenic response involving the PA/plasmin system.

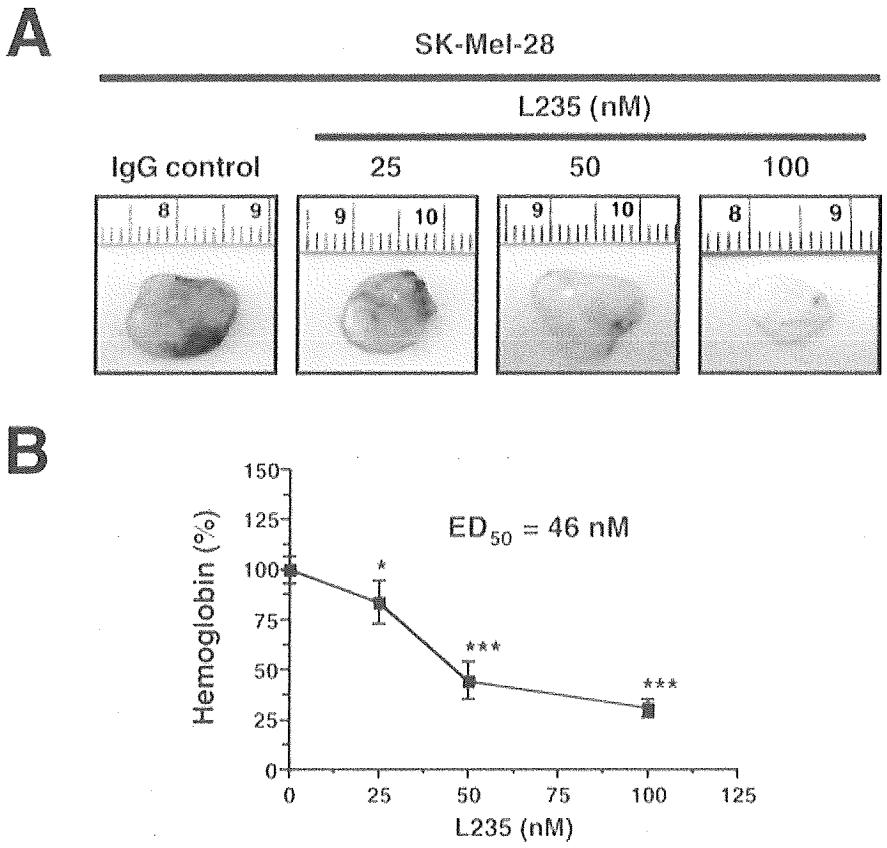

Fig. 8 L235 inhibits SK-Mel-28-induced neovascularization. SK-Mel-28-induced Matrigel™ plug *in vivo* angiogenesis assay containing, or lacking, the mAb L235, as described in the Materials and methods section. (**A**) Photos obtained from representative samples are shown. (**B**) Results were corrected for background Hb content measured in the Matrigel™ control condition and expressed as percentage inhibition compared with IgG control condition. $n = 12$ for IgG control condition, $n = 4$ for 25 and 100 nM conditions, $n = 9$ for 50 nM condition and the means ± SE are shown. Statistically significant differences compared with IgG control conditions are indicated as follows: $*P < 0.05$; $***P < 0.001$ (Student's *t*-test).

Subcutaneous, systemic truncated sMTf treatments inhibit SK-Mel-28-induced neovascularization

To antagonize the proangiogenic effects linked to MTf overexpression, as observed in the presence of SK-Mel-28 cells, we used a truncated soluble form of MTf (sMTf) to inhibit MTf-induced Matrigel™ implant neovascularization (Figure 9). First, mice were treated systemically four times (Days 0, 2, 4 and 6 post-implantation; s.c. injection) with either truncated sMTf (10, 20 and 40 mg/kg; total treatment) or with control R/H (Figure 9A). In a second set of experiments, Alzet® osmotic pumps (100 µl, 7-day delivery) containing either

truncated sMTf (10 and 20 mg/kg; total treatment) or control R/H were implanted subcutaneously on the same day as Matrigel™ plugs were implanted (Figure 9B). Quantitative analysis of angiogenesis indicated that s.c. systemic treatment with truncated sMTf antagonized neovascularization of the Matrigel™ implants, as demonstrated by a lower Hb content (Figure 9C). Moreover, continuous s.c. infusion of truncated sMTf using an Alzet® osmotic pump increased this antiangiogenic activity. In fact, the ED_{50} was reduced from 17 mg/kg (repeated s.c. injections) to 11 mg/kg when a continuous s.c. infusion was employed. These results show the efficacy of truncated sMTf in relatively low amounts as an inhibitor of the *in vivo* SK-Mel-28-stimulated angiogenesis.

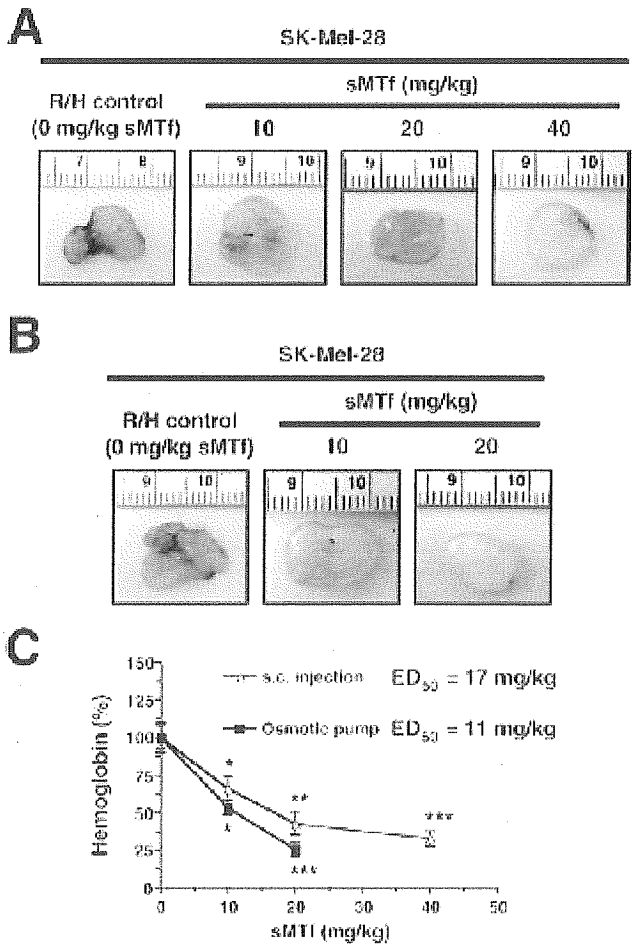

Fig. 9 Subcutaneous, systemic truncated sMTf treatments inhibit SK-Mel-28-induced neovascularization. (**A**) SK-Mel-28-induced Matrigel™ plug *in vivo* angiogenesis assay in nude mice treated systemically for 7 days with repeated s.c. injections of either R/H control solution or

truncated sMTf (10, 20 and 40 mg/kg/week), as described in the Materials and methods section. Photos obtained from representative samples are shown. (**B**) SK-Mel-28-induced Matrigel™ plug *in vivo* angiogenesis assay in nude mice treated systemically by s.c. infusion using an Alzet® osmotic pump of either R/H control solution or truncated sMTf (10 and 20 mg/kg/week), as described in the Materials and methods section. Photos obtained from representative samples are shown. (**C**) Hb content from Matrigel™ implants containing SK-Mel-28 cells in nude mice treated systemically for 7 days with repeated s.c. injections or Alzet® osmotic pump s.c. infusion of truncated sMTf. Results were corrected for background Hb content measured under the Matrigel™ control conditions and expressed as percentage inhibition compared with R/H control condition. $n = 7$ for s.c. injection R/H control condition, $n = 5$ for 10 and 40 mg/kg/week conditions, $n = 7$ for 20 mg/kg/week condition, $n = 3$ for each Alzet® osmotic pump conditions and the means ± SE are shown. Statistically significant differences compared with R/H control condition are indicated as follows: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ (Student's *t*-test).

Pharmacokinetic characteristics of truncated sMTf in mice

To assess the pharmacokinetic characteristics of truncated sMTf in mice, blood drug levels was studied in female athymic Crl:CD-1®-nuBR nude mice. The mean blood concentrations of truncated sMTf after a single 5 mg/kg s.c. injection or a continuous 20 mg/kg s.c. infusion using a Alzet® osmotic pump were measured (Figure 10). The pharmacokinetic parameters derived from these data are summarized in Table 2. After a single s.c. injection, truncated sMTf appeared rapidly in the circulation attaining a concentration maximum (C_{max}) of 41.0 ± 8.7 nM at 3 h. Next, using an Alzet® osmotic pump, truncated sMTf appeared more slowly in the circulation compared with the single s.c. injection and gained a C_{max} of 7.3 ± 1.9 nM at 144 h (Day 6). The terminal pharmacokinetics of truncated sMTf after a single s.c. administration were characterized by a slow elimination phase with a final steady state concentration (C_{last}) of 4.0 ± 1.5 nM, compared with 6.1 ± 1.6 nM when truncated sMTf is subcutaneously infused using an Alzet® osmotic pump. Also, the terminal half-life ($t_{1/2}$) of truncated sMTf was ~7.6 ± 0.9 h. The high exposure of the vasculature to truncated sMTf was also reflected by the high area under the curve from 0.02 h to infinity ($AUC_{0.02-\infty}$) of 476.1 ± 67.7 nM·h for a single injection and 928.0 ± 59.8 nM·h for an Alzet® osmotic pump infusion. In addition, the truncated sMTf quantity delivered by an Alzet® osmotic pump infusion represents ~3.5-fold the quantity of truncated sMTf delivered by a single s.c. injection. Thus, these results demonstrate that over a 7-day period treatment, the $AUC_{0.02-\infty}$ obtained with an Alzet® osmotic pump (928.0

nM·h) infusion is ~51% of that observed during a single s.c. injection (1808.8 nM·h). Furthermore, after a single s.c. dosing with 2.5 and 10 mg/kg of truncated sMTf, a dose-proportional relationship was observed in blood. Overall, these results show that s.c. dosing of truncated sMTf can achieve blood concentrations that are in the same order as those required for the inhibition of *in vitro* HUVEC tubulogenesis.

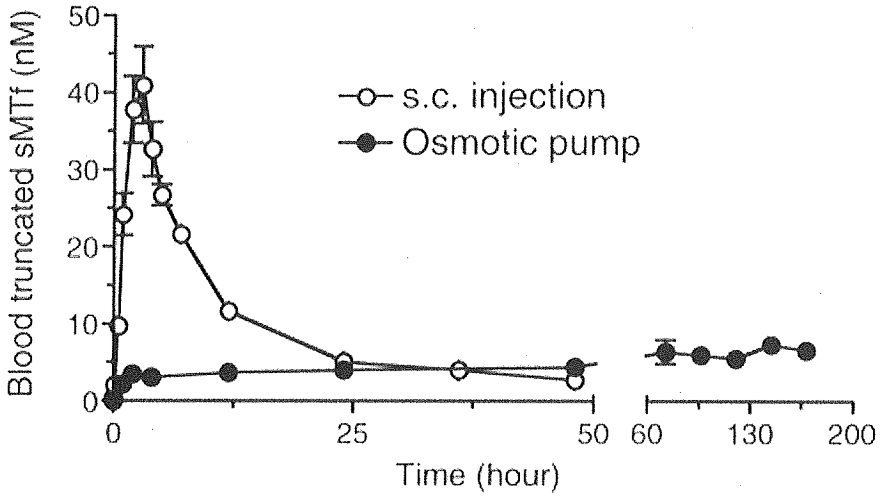

Fig. 10 Blood concentrations of truncated sMTf in nude mice. Female athymic Crl:CD-1®-nuBR nude mice received a single dose (open circle) of 5 mg/kg truncated sMTf in aqueous R/H solution by s.c. injection or by 7-day continuous s.c. infusion (closed circle) of 20 mg/kg truncated sMTf using an Alzet® osmotic pump, as described in the Materials and methods section. At the allotted times, blood was collected and the concentration of the compound was quantified by measuring blood-associated radioactivity using a gamma counter. Results were corrected for background radioactivity measured in untreated mice and expressed as truncated sMTf in blood (nM). The pharmacokinetic parameters derived from these data are summarized in Table 2. For each conditions, $n = 3$ and the means ± SE are shown.

Table 2 Pharmacokinetic parameters for truncated sMTf in blood after s.c. administration to female athymic Crl:CD-1®-nuBR nude mice

Administration route	Truncated sMTf parameters[a]			
	t_{max} (h)	C_{max} (nM)	C_{last} (nM)	$AUC_{(0.02-\infty)}$ (nM·h)
Single s.c. injection (5 mg/kg)	3.0 ± 0.6	41.0 ± 8.7	4.0 ± 1.5	476.1 ± 67.7
Continuous s.c. infusion (20 mg/kg/week)	144.0 ± 36.7	7.3 ± 1.9	6.1 ± 1.6	928.0 ± 59.8

[a]Areas under the curve from 0.02 h to infinity ($AUC_{0.02-\infty}$) were calculated using mean values. C_{max} (maximum concentration), t_{max} (time to maximum concentration) and C_{last} (steady-sate concentration) were determined by inspection of the data using GraphPad Prism. Data are expressed as mean ± SE ($n = 3$).

Conclusion: The results presented hereinabove demonstrate that sMTf treatment clearly decreases tumor development *in vivo*. Further, sMTf treatment decreases the angiogenesis stimulated by growth factors and leads to efficient growth inhibition of subcutaneous U-87 MG and NCI-H460 cells. It is further demonstrated that sMTf interferes with angiogenesis induced by growth factors like those express in high levels in tumor cells in very aggressive glioblastomas. Further, constant delivery of sMTf into nude mice with Alzet micro-osmotic pumps contributed to a significant reduction in the growth of subcutaneous U-87 MG-derived tumor. Consequently, since U-87 cells do not express mMTF, it is thus concluded that sMTf treatment exerts anti-angiogenic and anti-tumor activities not only in a MTF-expressing environment, but also on cells not expressing mMTF. In addition, the reduction of SK-Mel-28 cell metastasis in the lung observed with MTf-silenced cells indicates that MTf is involved during melanoma cell lung invasion.

Furthermore, *in vivo* antiangiogenic efficacy data correlate with the capacity of truncated sMTf to attain blood levels that are (i) in excess of *in vitro* concentration values needed for the inhibition of HUVEC tubulogenesis; (ii) sufficient to almost totally block the MTf-induced HUVEC tubulogenesis; (iii) in excess of *in vivo* sMTf concentration measured in human serum; and (iv) sufficient to demonstrate antiangiogenic effects in a bFGF-, VEGF- and cell-driven Matrigel™ implant neovascularization model. Also, the use of Alzet® osmotic pump infusion technique provided a higher therapeutic efficacy as compared with repeated s.c. injections, emphasizing the advantages of constant infusion rather than pulse-dose drug inputs for antiangiogenic effects. Our results are also consistent with data on angiostatin dosing patterns in mice, where continuous infusion of the drug had a dramatically improved antiangiogenic effect over twice-daily administration of

the same dose. Moreover, the final steady state concentration of truncated sMTf is greater than the concentration of endogenous sMTf present in human serum (0.017–0.046 nM), indicating that serum truncated sMTf modulation could be achieved. On the basis of this favorable preclinical profile, truncated sMTf can now be considered an antiangiogenesis treatment modality in clinical testing.

8. The results presented above and produced according to the teaching of the present invention clearly proves that that the present invention have clinical relevance and in addition, that the *in vitro* results disclosed in the present application do not diverge from *in vivo* responses. The anti-tumoral activity of sMTf of the present invention is demonstrated *in vitro* and *in vivo*.

9. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both (18 U.S.C. Sec. 1001), and may jeopardize the validity of the application of any patent issuing thereon.

Signed  Dated: March 13, 2008

Michel Demeule

Curriculum vitae

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ACADEMIC FORMATION

Ph. D. (Physiology)	Université de Montréal	1989-1992
M. Sc. (Chemistry)	Université du Québec à Montréal	1986-1988
B. Sc (Biochemistry)	Université du Québec à Montréal	1983-1986

EXPERIENCE

1) Associate-Director Research at Angiochem (2006-)

- Supervision of reasearch projects associated to the development of Angiochem platform. Characterization of Angiochem brain delivery system in new therapeutical approaches. Management of the Research group (drug conjugation, biological assays and *in vivo* models). Design and evaluation of new molecules for the treatments of brain diseases.

2) Project leader (1993-2006)

Laboratory of Molecular Medicine
UQAM and Ste.-Justine Hospital

- Supervision of research projects related to the blood-brain barrier, multidrug resistance and to the utilization of active natural products in cancer prevention and treatments.

- Supervision of Ph.Ds., research assistants, technicians and graduate students.

- Involvement in collaborations with biotechnological and pharmaceutical companies including Conjuchem, Aeterna, Novartis, Sandoz and AstraZeneca.

- Redaction of manuscripts, book chapter and research grants.

2) Ph. D. studies (1989-1992)

Subject: Molecular interactions of CsA with kidney membranes. Research director: Dr Richard Béliveau

3) M. Sc. studies (1986-1988)

Subject: Characterization of kidney cortex transporters: Determination of their molecular mass by radiation inactivation. Research director: Dr Richard Béliveau

4) Professor assistant
Department of biochemistry-chemistry, UQAM

- Biochemistry Techniques I (CHI-3510) Autumn 87
- Biochemistry Techniques II (CHI-3532) Autumn 86

OTHER INFORMATION

1) Domains of expertise: Cancer, blood-brain barrier, membrane transport, angiogenesis, structure-function studies of proteins, biospecific interaction analysis (BIAcore), enzymology, electrophoresis, Western blots, photoaffinity labeling, high performance liquid chromatography, protein purification by chromatography (FPLC-AKTA).

2) Computer knowledge: Macintosh/IBM (Microsoft Word, Excel, Kaleaidagraph, Photoshop, Statview, etc.)

2) Spoken languages: French, English

3) Written languages: French, English

SCOLARSHIPS

GRTM and Sandoz fellow	1991-1992
FRSQ and Sandoz fellow	1990-1991
FCAR fellow	1989-1990
GRTM fellow	1987-1989

PUBLICATIONS (see appendices)

1) Peer review papers: 56

2) Book chapter: 3

3) Patents: 4

APPENDICES

Publications in the last six years:

1. Demeule M., A. Regina, C. Che, J. Poirier, T. Nguyen, R. Gabathuler, J.P. Castaigne and R. Béliveau (2008) Identification and Design of New Peptides as a Drug Delivery System for the Brain. *J. Pharmacol. Exp. Ther.* 324 :1064-1072.

2. Rolland Y., M. Demeule, J. Michaud-Levesque and R. Béliveau (2007) Inhibition of tumor growth by a truncated and soluble form of melanotransferrin. *Exp. Cell Res.* 313:2910-2919

3. Michaud-Levesque J., M. Demeule and R. Béliveau. Plasminogen-dependent internalization of soluble melanotransferrin involves the low-density lipoproptein receptor-related protein and annexin II. *Biol. Chem.* 388:747-754.

4. Barakat S., M. Demeule, A. Pilorget, A. Regina, D. Gingras, L.G. Baggetto and R. Béliveau (2007) Modulation of p-glycoprotein function by caveolin-1 phosphorylation. *J. Neurochem.* 101:1-8.

5. Pilorget A., M. Conesa, S. Sarray, J. Michaud-Levesque, S. Daoud, K.S. Kim, M. Demeule, J. Marvaldi, M. El Ayeb, N. Marrakchi, R. Béliveau and J. Luis (2007) Lebectin, a Macrovipera lebetina venom-derived C-type lectin, inhibits angiogenesis both in vitro and in vivo. *J. Cell. Physiol.* 211:307-315.

6. Pilorget A., M. Demeule, S. Barakat, J. Marvaldi, J. Luis and R. Béliveau (2007) Modulation of P-glycoprotein function by sphingosine kinase-1 in brain endothelial cells. *J. Neurochem.* 100:1203-1210.

7. Bertrand Y., M. Demeule, J. Michaud-Levesque and R. Béliveau (2007) Melanotransferrin induces human melanoma SK-Mel-28 cell invasion in vivo. *Biochem. Biophys. Res. Commun.* 353:418-423.

8. Michaud-Levesque J., M. Demeule and R. Béliveau (2007) In vivo inhibition of angiogenesis by a soluble form of melanotransferrin. *Carcinogenesis* 28:280-288.

9. Bertrand Y., M. Demeule, G.E. Rivard and R. Béliveau (2006) Stimulation of tPA-dependent provisional extracellular fibrin matrix degradation by human recombinant soluble melanotransferrin. *Biochim. Biophys. Acta* 1763:1024-1030.

10. Barthomeuf C., M. Demeule, J. Grassi, A. Saidkhodjaev and R. Béliveau (2006) Conferone from Ferula schtschurowskiana enhances vinblastine cytotoxicity in MDCK-MDR1 cells by competitively inhibiting P-glycoprotein transport.Planta Med. 72:634-639.

11. Rolland Y., M. Demeule and R. Béliveau (2006) Melanotransferrin stimulates tPA-dependent activation of plasminogen in endothelial cells leading to cell detachment. in *Bioch. Biophys. Acta.* 1763:393-401.

12. Michaud-Lévesque J., M. Demeule and R. Béliveau (2007) In vivo angiogenesis inhibition by a soluble form of melanotransferrin. Accepted for publication in *Carcinogenesis* 28:280-288.

13. Michaud-Lévesque J., M. Demeule and R. Béliveau (2005) Stimulation of cell surface plasminogen activation by membrane-bound melanotransferrin : a key phenomenon for cell invasion. *Exp. Cell. Res.* 15:479-490

14. Barthomeuf C., J. Grassi, M. Demeule, C. Fournier, D. Boivin and R. Béliveau (2005) Inhibition of P-glycoprotein transport function and reversion of MDR1 multidrug resistance by cnidiadin. *Cancer Chemother Pharmacol.*. 56:173-181.

15. Demeule M., B. Annabi, J. Michaud-Lévesque , S. Lamy and R. Béliveau (2005) Dietary prevention of cancer: Anticancer and Antiangiogenic Properties of Green Tea Polyphenols. *Medicinal Chemistry Reviews* 2:49-58.

16. Michaud-Lévesque J., Y. Rolland, M. Demeule, Y. Bertrand and Béliveau R. (2004) Inhibition of endothelial cells migration and tubulogenesis by exogenous human recombinant soluble melanotransferrin : involvement of the uPAR/LRP plasminolytic system. *Bioch. Biophys. Acta.* 1743:243-253.

17. Boivin D., M. Provencal, S. Gendron, D. Ratel, M. Demeule, D. Gingras and R. Beliveau (2004) Purification and characterization of a stimulator of plasmin generation from the antiangiogenic agent Neovastat: identification as immunoglobulin kappa light chain. *Arch. Biochem. Biophys.* 431:197-206.

18. Demeule M., M. Brossard, S. Turcotte, A. Regina, J. Jodoin and R. Béliveau (2004) Diallyl dusulfide a chemopreventive agent in garlic induces multidrug-resistance associated protein 2 expression *Biochem. Biophys. Res. Comm.* 324: 937-945.

19. Demeule M., A. Regina, B. Annabi, Y. Bertrand, M. Bojanowki and R. Béliveau (2004) Brain endothelial cells as pharmacological targets in brain tumors. *Mol. Neurobiol.* 30:157-184.

20. Régina A., J. Jodoin, P. Khoueir, Y. Rolland, F. Berthelet, R. Moumdjian, L. Fenart, R. Cecchelli, M. Demeule and R. Béliveau (2004) Down-regulation of caveolin-1 in glioma vasculature: modulation by radiotherapy. *J Neurosci. Res.* 75:291-299.

21. Gingras D., D. Labelle, C. Nyalendo, D. Boivin, M. Demeule, C. Barthomeuf and R. Béliveau (2004) The antiangiogenic agent Neovastat (AE-941) stimulates tissue plasminogen activator activity. *Invest. New Drugs* 22:17-26.

22. Desrosiers R.R., Y. Bertrand, Q.T. Nguyen, M. Demeule, R. Gabathuler, M.L. Kennard, S. Gauthier and R. Béliveau (2003) Expression of melanotransferrin isoforms in human serum: relevance to Alzheimer's disease.*Biochem. J.* 374:463-471.

23. Demeule M., Y. Bertrand, J. Michaud-Lévesque, J. Jodoin, Y. Rolland, R. Gabathuler, and R. Béliveau (2003). Plasminogen activation by urokinase plasminogen activator: A potential role for melanotransferrin (p97) in cell migration. *Blood* 102:1723-1731.

24. Jodoin J., M. Demeule, L. Fenart, R. Cecchelli, S. Farmer, J.L. Linton, C.F. Higgins and R. Béliveau (2003) P-glycoprotein in blood-brain barrier endothelial cells: interaction and oligomerization with caveolins. *J. Neurochem.* 87:1010-1023.

25. Laplante A., D. Liu, M. Demeule, B. Annabi, G.F. Murphy, P. Daloze, H. Chen, and R. Béliveau (2003) Modulation of matrix gelatinases and MMP-activating process in acute kidney rejection. Transpl. Int. 16:262-269.

26. Régina A., M. Demeule, A. Bérubé, R. Moumdjian, F. Berthelet, and R. Béliveau (2003). Differences in multidrug resistance phenotype and matrix metalloproteinases activity between endothelial cells from normal brain and glioma. J. Neurochem. 84:316-324.

27. Demeule M., A. Régina, J. Jodoin, A. Laplante, C. Dagenais, F. Berthelet, A. Moghrabi, and R. Béliveau (2002). Drug transport to the brain: key roles for

the efflux pump P-glycoprotein in the blood-brain barrier. *Vascul. Pharmacol.* 38:339-348.

28. Laplante A., M. Demeule, G.F. Murphy, and R. Béliveau (2002). Interaction of immunosuppressive agents rapamycin and its analogue SDZ-RAD with endothelial P-gp. *Transplant. Proc.* 34:3393-3395.

29. Demeule M., J. Poirier, J. Jodoin, Y. Bertrand, R.R. Desrosiers, C. Dagenais, T. Nguyen, J. Lanthier, R. Gabathuler, M. Kennard, W.A. Jefferies, D. Karkan, S. Tsai, L. Fenart, R. Cecchelli, and R. Béliveau (2002). High transcytosis of melanotransferrin (P97) across the blood-brain barrier. *J. Neurochem.* 83:924-933.

30. Lanthier J., A. Bouthillier, M. Lapointe, M. Demeule, R. Beliveau, and R.R. Desrosiers (2002). Down-regulation of protein L-isoaspartyl methyltransferase in human epileptic hippocampus contributes to generation of damaged tubulin. *J. Neurochem.* 83:581-591.

31. Demeule M., J. Michaud-Lévesque, B. Annabi, D. Gingras, D. Boivin, J. Jodoin, S. Lamy, Y. Bertrand, and R. Béliveau (2002). Green tea catechins as novel antitumor and antiangiogenic compounds. *Curr. Med. Chem.-Anti-Cancer Agents* 2:441-463.

32. Jodoin J., M. Demeule, and R. Béliveau (2002). Inhibition of the multidrug resistance P-glycoprotein activity by green tea polyphenols. *Biochim. Biophys. Acta.* 1542:149-159.

33. Régina A., M. Demeule, A. Laplante, J. Jodoin, C. Dagenais, F. Berthelet, A. Moghrabi, and R. Béliveau (2001). Multidrug resistance in brain tumors: roles of the blood-brain barrier. *Cancer Metastasis Rev.* 20:13-25.

34. Demeule M., D. Shedid, É. Beaulieu, R.F. Del Maestro, A. Moghrabi, P.B. Ghosn, R. Moumdjian, F. Berthelet, et R. Béliveau (2001). Expression of multidrug-resistance P-glycoprotein (MDR1) in human brain tumors. *Int. J. Cancer* 93:62-66.

35. Demeule M., M. Labelle, A. Régina, F. Berthelet, and R. Béliveau (2001). Isolation of endothelial cells from brain, lung, and kidney: expression of the multidrug resistance P-glycoprotein isoforms. Biochem. Biophys. *Res. Commun.* 281:827-834.

Book chapters :

1. Turcotte S., M. Demeule, A. Régina, C. Fournier, J. Jodoin, A. Moghrabi and R. Béliveau (2006) The blood-brain barrier: roles of the multidrug resistance transporter P-glycoprotein. Book chapeter for the Blood-Brain Barriers, Ed. by Rolf Dermietzel, David C. Spray and Maiken Nedergaard, Wiley-VCH Verlag GmbH & Co, KGaA, Winheim.

2. Laplante A., J. Jodoin, M. Demeule, and R. Béliveau (2000). P-glycoprotein : a key player in the blood-brain barrier. *Recent Res. Devel. Biochem.* 2:51-64.

3. Béliveau R., M. Demeule, É. Beaulieu, and L. Jetté (1998). Chemotherapy and Chemosensitization in an Introduction to the Blood-Brain Barrier. Cambridge University Press, W.M. Pardridge Ed. 301-307.

Patents :

1. Béliveau R., Demeule M., Yang J., Kennard M. L., Gabathuler R. Compositions and methods for modulating blood-brain barrier transport. Patent number: WO03009815 (2003-02-06).

2. Béliveau R., Demeule M. A method for transporting a compound across the blood-brain barrier. Patent number: WO2004060403 (2004-07-22).

3. Béliveau R., Demeule M., Bertrand Y., Michaud-Levesque J., Rolland Y., Jodoin J. Compound and method for regulating plasminogen activation and cell migration. Patent number: WO2004099410 (2004-11-18).

4. Béliveau R., Demeule M., Barakat S., Michaud-Levesque J. Compounds and methods stimulating P-glycoprotein function. (new provisional application for patent).